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RESPONSE OF BITTER VETCH LINES TO SALT STRESS

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Abstract

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This research was carried out in a greenhouse to evaluate the response of bitter vetch (*Vicia ervilia* (L.) Willd) to increasing salinity levels. Saline irrigation waters with NaCl concentrations of 0, 2.5, 5, 7.5 and 10 dS m^{-1} were applied to investigate the salt tolerance of the plants. Shoot and root length (cm), shoot and root fresh and dry weight (g/plant), Na^+, K^+, Cl^- (%) content of dry matter and Na^+:K^+ ratio, crude protein, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) parameters were investigated. Research results revealed reduced growth parameters with elevated salt levels. While Na^+, Cl^- contents of shoot and root dry matter, crude protein and crude ash percentage increased with elevated NaCl concentrations, a decrease was observed in ADF and NDF.

Key words: bitter vetch, *Vicia ervilia* (L.) Willd, NaCl, salt stress, ion accumulation

Introduction

Salinity is a major abiotic stress that limits crop productivity. Salinity problem is encountered naturally in arid and semiarid regions of the world where annual rainfall is limited for leaching excessive salt and also encountered in irrigated areas with both poor quality irrigation water and poor irrigation management (Ünlükara et al., 2008; Day et al., 2008; Abed-Alrahman et al., 2005). Several plant species are sensitive to salinity with negative effect on growth. Growth inhibition has been linked to osmotic effects that provoke water deficit. Excess salinity may also cause toxicity and mineral deficiencies (Praxedes et al., 2010). To solve the salinity problem, salts can be leached from the soil profile by irrigation. However, the cost and availability of irrigation water make the leaching unfeasible for large size lands. This has led to researcher to investigate about salt tolerant or salt resistant genotypes (Abdel-Ghani, 2009).

Bitter vetch is annual crop grown in Southern Europe, Western and Central Asia, and North Africa for forage and grain. The crop can be grown on very shallow, alkaline soils where other grains such as corn and soybean do not grow successfully. It improves soil fertility by fixing atmospheric nitrogen (Serin and Tan, 2001; Larbi et al., 2011). Previous bitter vetch studies focused on grain and forage yield and quality, in vitro propagation rather than salinity stress (Larbi et al., 2011; Etadali et al., 2011; Ayan et al., 2006; Erdoğan et al., 2005). Therefore, this study was conducted to search the growth and physiological responses of bitter vetch lines under elevated salinity levels.

Materials and Methods

This study was conducted at Erciyes University Agricultural Faculty Field Crops Department of Kayseri, Turkey. The bitter vetch lines obtained from Prof. Dr. Hayrettin Ekiz.

Experiments were carried out under greenhouse conditions in a completely randomized block design with three replications between April and May of the year 2010. The soil used in the pot experiment, taken from university campus area, was air dried and sieved through 4 mm sieve. Soil analysis showed that soil contained 51% sand, 33% silt and 15% clay, pH of 7.5, 0.9% organic matter, 1.48 % lime, 0.084% total nitrogen, 20.94 kg P_2O_5/da, 1.91 me/100 g exchangeable K^+, 0.75 me/100g exchangeable Na^+, 16.6 me/100g exchangeable Ca^{+2}, 2.63 me/100 g exchangeable Mg^{+2}, electrical con-

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ductivity (1:2.5) of 0.353 dS/m, field capacity of 18.31% and permanent wilting point of 9.49%.

Pots were filled with 2.5 kg soil. Bitter vetch seeds were sown at a rate of 10 seeds per pot at 2 cm depth. The pots were irrigated with tap water during the initial 10 days (until emergence occurs). After emergence, seedlings were thinned to five healthy plants per pot. Saline water was applied once every two days at step-wise increase (¼, ½, ¾ strengths) until the target irrigation water salinity was reached. Saline irrigation waters with NaCl concentrations of 0, 2.5, 5, 7.5 ve 10 dS m^{-1} were applied, but plants were not alive above 5 dS m^{-1} All irrigation treatments were applied based on soil field capacity plus 20% leaching fraction (Ünlükara et al., 2008). Plants were harvested at 50% flowering stage. Shoot and root lengths and fresh weights were measured. After the harvest, shoots and roots were rinsed in distilled water to remove salt and soil. Samples were dried in oven at 70°C for 48h and dry weights were determined. The oven-dried root and shoot tissues were ground to a fine powder and 500 mg of the sample was transferred to a digestion flask containing 6 ml acid mixture of HNO_3 and $HClO_4$ at 4:1(v/v) ratio. The flask was heated gently on a heat block, cooled and diluted by adding distilled water. Sodium and potassium analyses were performed by flame photometric method. Chloride contents were determined by using a titrimetric method (Kacar and İnal, 2008).

The ash content was determined by igniting the samples in a muffle furnace at 525°C for 8h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). The crude protein was calculated as Nx6.25. The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) of samples were analyzed with the ANKOM fiber analyzer using reagents described by Van Soest (1963) and Van Soest and Robertson (1985), respectively.

Randomized block design with two factors were used to carry out the experiments. The first factor was lines and the second factor was NaCl levels. For all investigated parameters, an analysis of variance (ANOVA) was performed by using SPSS 16 for Windows. Significance of differences among the mean values were tested by LSD test ($P<0.05$).

Results and Discussion

Summary of ANOVA, LSD values and 2 way interaction values were shown in Table 1. While shoot lenght, shoot and root dry weight were significantly affected by lines and salinity levels separately, root length, shoot and root fresh weight were affected by the interaction between lines and salinity levels (Table 1). Increased NaCl concentrations decreased shoot length of all lines. The longest and shortest roots were obtained respectively from line 2 with 22.83 cm under 2.5 dS/m NaCl level and from line 10 with 15.36 cm under 5 dS/m NaCl level. In general, root lengths, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight of the lines were influenced by salt stress especially at NaCl stress of 5 dS/m. The decreases in these parameters were more pronounced in line 9 and 10 than in line 1, 2 and 8.

In general, salinity treatment especially at NaCl stress of 5 dS/m resulted in decreasing plant growth. Reduction in plant growth by means of salt stress was also reported by various scientists (Okçu et al., 2005; Atak, 2006; Ateş and Tekeli, 2007; Day et al., 2008; Bilgin et al., 2008). Beyaz et al. (2011), Mer at al. (2000) and Tunçtürk et al. (2011). Abed Alrahman et al. (2005) and Abdel-Ghani (2009) reported that reduction in growth with increasing salinity could be attributed to salinity induced water deficit, ion toxicity associated with excessive uptake particularly of Na^+ and Cl^-, and nutritional imbalance as a result of depressed uptake, shoot transport and impaired internal distribution of minerals especially of K^+ and Ca^{+2} .

Effects of lines, NaCl levels and interaction on shoot and root, Na^+, K^+, Cl^- content and Na^+:K^+ ratio and mean values were summarized in Table 2 and Figure 1. Na^+ and Cl^- contents of both shoots and roots increased with elevated NaCl concentrations. The trend of Na^+ accumulation in the lines was different from that of K^+ accumulation. There was less increase or decrease in K^+ accumulation in both of shoot and root. Due to high increase of Na^+ content, Na^+:K^+ ratio of shoots and roots increased with elevated NaCl concentrations.

The work by Essa (2002) with *Glycine max*, Misra and Dwiedi (2004) with *Phaseolus aureus*, Murillo-Amador et al. (2002) with *Vigna unguiculata* indicate that Na^+ and Cl^- content of plant parts increased with elevated NaCl practices. These findings are also in good agreement with the results of current study. Parallel to increasing NaCl, Na^+ accumulation and low increase or decline in K^+ content could have been resulted from competition between Na^+ and K^+ ions at absorptive sites of the plant roots. The results of this study are also in conformity with Kiliç et al. (2008), Meloni et al. (2008), Misra and Dwivedi (2004). Several reports indicate that the ability of plants to maintain high internal K^+ concentrations determined the salt tolerance of a plant (Rehman et al., 2000; Rejili et al., 2007; Kiliç et al., 2008). The Na^+:K^+ ratio also plays an extremely significant role in salinity tolerance because since the Na^+:K^+ ratio is related to higher salt tolerance (Alian et al., 2000; Oz et al., 2011).

NDF were significantly affected by lines and salinity levels, whereas crude protein ADF, NDF and crude ash we-

Table 1
Effect of different salinity levels on some plant growth parameters of five bitter vetch lines

Line	Salinity, dSm^{-1}	Shoot length, cm	Root length, cm	Fresh shoot weight, g $plant^{-1}$	Fresh root weight, g $plant^{-1}$	Dry shoot weight, g $plant^{-1}$	Dry root weight, g $plant^{-1}$
Line 1	Control	25 ± 0.65	19.47 ± 0.89	2.16 ± 0.14	1.45 ± 0.4	0.46 ± 0.1	0.25 ± 0.04
	2.5	24.61 ± 1.42	21.49 ± 1.73	2.46 ± 0.24	1.85 ± 0.08	0.5 ± 0.03	0.27 ± 0.02
	5.0	21.25 ± 2.61	18 ± 1.48	1.81 ± 0.19	1.27 ± 0.33	0.39 ± 0.07	0.21 ± 0.07
	Mean	23.62	19.66	2.14	1.52	0.45	0.25
Line 2	Control	23.11 ± 1.01	22.42 ± 1.41	2.13 ± 0.13	1.66 ± 0.13	0.42 ± 0.03	0.22 ± 0.01
	2.5	22.49 ± 0.84	22.83 ± 0.68	2.49 ± 0.13	1.97 ± 0.27	0.46 ± 0.03	0.23 ± 0.03
	5.0	19.14 ± 0.48	21.58 ± 1.03	2.08 ± 0.06	1.49 ± 0.18	0.39 ± 0.02	0.16 ± 0.04
	Mean	21.58	22.27	2.24	1.71	0.42	0.2
Line 8	Control	19.71 ± 0.95	19.08 ± 0.52	2.06 ± 0.34	1.53 ± 0.09	0.4 ± 0.06	0.19 ± 0.02
	2.5	19.93 ± 0.6	20.1 ± 1.35	2.18 ± 0.21	1.47 ± 0.11	0.4 ± 0.04	0.17 ± 0.01
	5.0	18.26 ± 0.57	20.06 ± 0.87	1.84 ± 0.27	1.42 ± 0.14	0.34 ± 0.02	0.15 ± 0.03
	Mean	19.3	19.75	2.02	1.47	0.38	0.17
Line 9	Control	26.12 ± 1.3	19.85 ± 0.31	2.76 ± 0.25	1.85 ± 0.12	0.52 ± 0.08	0.15 ± 0.02
	2.5	25.12 ± 1.45	19.24 ± 0.51	2.03 ± 0.22	1.53 ± 0.15	0.44 ± 0.05	0.13 ± 0.02
	5.0	21.63 ± 1.04	15.89 ± 1.27	1.65 ± 0.16	0.9 ± 0.06	0.35 ± 0.06	0.11 ± 0.02
	Mean	24.29	18.33	2.15	1.42	0.44	0.13
Line 10	Control	20.78 ± 0.66	18.75 ± 0.44	2.08 ± 0.18	1.89 ± 0.18	0.41 ± 0.06	0.18 ± 0.02
	2.5	19.75 ± 1.23	18.72 ± 0.25	1.94 ± 0.11	1.86 ± 0.14	0.41 ± 0.02	0.18 ± 0.03
	5.0	16.57 ± 0.65	15.36 ± 0.71	1.38 ± 0.08	0.97 ± 0.03	0.26 ± 0.02	0.11 ± 0.01
	Mean	19.03	17.61	1.8	1.57	0.36	0.16
Mean	control	22.94	19.92	2.24	1.68	0.44	0.2
	2.5 EC	22.38	20.48	2.22	1.74	0.44	0.2
	5 EC	19.37	18.18	1.75	1.21	0.35	0.15
ANOVA		Sig. lsd	Sig. lsd	Sig. lsd	Sig. lsd	Sig. lsd	Sig. lsd
Line		** 1.11	**	**	*	** 0.053	** 0.03
NaCl level		** 0.86	**	**	**	** 0.049	** 0.24
Line x NaCl		ns	** 1.67	** 0.33	** 0.31	ns	ns

Data represent mean±SD of three replicates; LSD values, P<0.05, df=30,
**, *significant at the 0.01 probability levels, ns: non-significant

Table 2
Summary of ANOVA for the effect of lines and NaCl levels on ion accumulation of shoot and root

Parameters	Lines	NaCl	Lines x NaCl	Parameters	Lines	NaCl	Lines x NaCl
Na^+ in shoot	ns	**	ns	Na^+ in root	**	**	**
K^+ in shoot	**	**	ns	K^+ in root	**	ns	**
Cl^- in shoot	**	**	*	Cl^- in root	**	**	*
Na^+:K^+ in shoot	ns	**	ns	Na^+:K^+ in root	**	**	**

**, *significant at the 0.01 and 0.05 probability levels, respectively, ns: non-significant

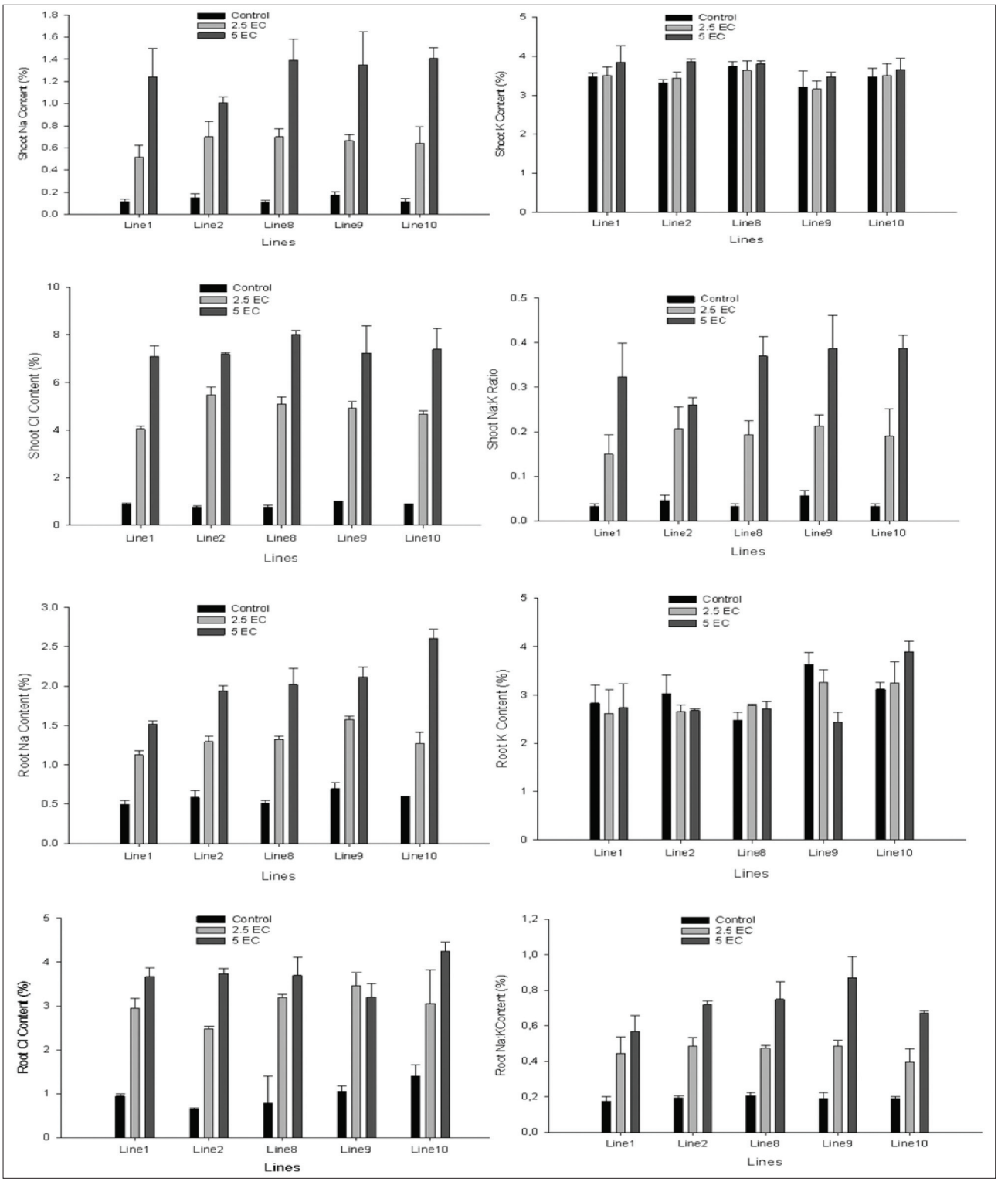

Fig. 1. Interactive effect of bitter vetch lines and NaCl levels on ion accumulation

a) Na^+ in shoot (LSD_{NaCl}: 0.10), b) K^+ in shoot (lsd_{lines}:0.22, lsd_{NaCl}:0.17), c) Cl^- in shoot (lsd_{int}:0,69), d) Na^+/K^+ in shoot (lsd_{NaCL}:0,003), e) Na^+ in root (lsd_{int}: 0.15), f) K^+ in root (lsd_{int}:0,49), g) Cl^- in root (lsd_{int}:0,54), h) Na^+/K^+ in root (lsd_{int}:0,091), $p < 0,05$, df=30

re affected by the interaction between lines and salinity levels (Table 3). The highest percentage of crude protein was observed from line 8 in control treatment and the lowest percentage obtained from line 8 in 2.5 dS/m salinity levels. Salinity had differential effects depending upon the lines. Crude protein percentage increased with salinity in lines 1, 2, 9 and 10 but the opposite was observed for line 8 and 10. Kumar et al. (2010) and Abed Alrahman et al. (2005) also reported inhibitory effect of salinity on leaf protein in oat and Abed Alrahman et al. (2005) in cucumber. Conversely, the work of Suyama et al. (2007) with tall wheat grass, paspalum, creeping wildrye, bermudagrass and alfalfa revealed increasing protein percentages with increased salinity.

Table 3
Effect of different salinity levels on the percentage of crude protein, ADF, NDF and crude ash of five bitter vetch lines

Line	Salinity, dSm^{-1}	Crude protein, %	ADF, %	NDF, %	Crude ash, %
Line 1	Control	17.22 ± 1.27	24.14 ± 0.99	31.07 ± 0.37	10.59 ± 0.8
	2.5	17.48 ± 1.06	24.38 ± 0.54	29.15 ± 0.73	14.06 ± 0.36
	5	19.38 ± 0.41	23.44 ± 0.58	27.03 ± 0.9	15.46 ± 0.52
	Mean	18.03	23.99	29.08	13.37
Line 2	Control	17.44 ± 0.52	25.46 ± 0.49	29.98 ± 0.55	11.44 ± 0.51
	2.5	17.91 ± 0.44	24.85 ± 1.31	28.72 ± 1.51	15.11 ± 1.21
	5	19.04 ± 0.96	21.53 ± 1.68	29.95 ± 0.09	18.08 ± 0.5
	Mean	18.13	23.95	29.55	14.88
Line 8	Control	21.24 ± 1.37	24.52 ± 0.5	31.61 ± 0.6	11.62 ± 0.92
	2.5	17 ± 1.05	23.35 ± 0.33	28.53 ± 0.76	16.6 ± 1
	5	18.4 ± 0.27	22.36 ± 0.29	28.86 ± 1.37	18.24 ± 0.61
	Mean	18.88	23.41	29.67	15.49
Line 9	Control	17.93 ± 1.16	29.02 ± 1.08	33.1 ± 0.79	11.85 ± 0.49
	2.5	17.96 ± 0.93	27.03 ± 0.25	32.89 ± 1.64	14.87 ± 0.33
	5	17.35 ± 0.48	24.22 ± 0.2	31.99 ± 2.75	18.26 ± 0.66
	Mean	17.75	26.76	32.66	14.99
Line 10	Control	18.86 ± 0.23	24.78 ± 0.88	29.62 ± 0.73	14.54 ± 0.4
	2.5	18.7 ± 0.62	23.69 ± 0.27	28.85 ± 0.58	15.83 ± 1.03
	5	19.82 ± 0.88	20.03 ± 0.96	28.14 ± 0.25	20.51 ± 0.96
	Mean	19.12	22.83	28.87	16.96
Mean	Control	18.54	25.58	31.08	12.01
	2.5	17.81	24.66	29.63	15.3
	5	18.8	22.32	29.19	18.11
ANOVA		Sig, lsd	Sig, lsd	Sig, lsd	Sig, lsd
Line		**	**	** 1.08	**
NaCl levels		**	**	** 0.83	**
Line x NaCl		** 1.43	** 1.35	Ns	** 1.23

Data represent mean±*SD* of three replicates; *LSD values,* P<0.05, *df*=30)
**, *significant at the 0.01 probability levels
NS: non-significant

While ADF and NDF percentages decreased under salinity, an increase was observed in crude ash percentage. Robinson et al. (2004) in different forages, Ben-ghedalia et al. (2001) in ryegrass also observed a decrease in NDF under salinity and results of current study are consistent with the results of these studies. Guerrero-Rodriguez et al. (2011) also observed increasing total and soluble ash concentrations with increasing salinity levels and reported different responses of species to salinity levels. Researchers observed an increase in ash content in lucerne leaves and in stems of both lucerne and white melilotus, but a decrease in white melilotus leaf.

Conclusions

This study demonstrated that under saline conditions, while Na^+ and Cl^- contents of shoots and roots increased excessively in bitter vetch, K^+ content decreased or increased slowly. Salt stress had significantly declined the plant growth especially at salinity level of 5 dS/m. While ADF and NDF decreased under salinity, an increase was observed in crude ash percentage. Results of this study revealed the line 9 and 10 as the relatively sensitive specifies to salinity.

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